Data Evaluation Report on the Acute Toxicity Effects of BAS 800 H (Saflufenacil) on Earthworms

PMRA Submission Number: 2008-0431
PMRA Document ID: 1547238 EPA MRID Number: 47127927

Data Requirement:	PMRA Data Code	9.2.3.1
	EPA DP Barcode	DP349851
	OECD Data Point	IIA 8.9.1
	EPA MRID	47127927
	EPA Guideline	OPPTS 850.6200

Test material: BAS 800 H **Purity:** 93.8%
Common name: Saflufenacil
Chemical name: IUPAC: N'-[2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydro-1(2H)-pyrimidinyl)benzoyl]-N-isopropyl-N-methylsulfamide
CAS name: Not Reported
CAS No.: Not Reported
Synonyms: None Provided

Primary Reviewer: John Marton **Signature:**
Staff Scientist, Cambridge Environmental, Inc. **Date:** 03/28/08

Secondary Reviewer: Teri S. Myers **Signature:**
Senior Scientist, Cambridge Environmental, Inc. **Date:** 04/08/08

Primary Reviewer: Anita Pease **Date:** 06/09/09
Senior Biologist, U.S. EPA

Secondary Reviewer: Janine Glaser **Date:** 06/09/09
HC-PMRA-EAD

Secondary Reviewer: Farzad Jahromi **Date:** 06/09/09
DEWHA-APVMA

Company Code BAZ
Active Code SFF
Use Site Category: 13 (terrestrial feed crops) and 14 (terrestrial food crops)
EPA PC Code 118203

CITATION: Vértesi, A. 2006. Acute Toxicity of BAS 800 H (Reg No. 4054449) on Earthworms (*Eisenia fetida*) in Artificial Soil with 5% Peat. Unpublished study performed by LAB International Research Center Hungary Ltd., H-8200 Veszprém, Szabadságpuszta. Laboratory report number 06/230-125G. Study sponsored by BASF Aktiengesellschaft, AP Agricultural Products Division, Agricultural Center Limburgerhof, D-67114 Limburgerhof. Study completed December 13, 2006.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to earthworms. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

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EXECUTIVE SUMMARY:

In an acute toxicity study, earthworms (*Eisenia fetida*) were exposed to BAS 800 H (Saflufenacil) for 14 days at concentrations of 0 (control), 31.3, 62.5, 125, 250, 500 and 1000 mg a.i./kg dw soil. The reference chemical used was 2-Chloroacetamide at concentrations of 2.5, 5, 10, 20, 40 and 80 mg a.i./kg dw soil. The LC_{50} was >1000 mg a.i./kg dw of soil. The EC_{50} was >1000 mg a.i./kg dw of soil. The NOAEC and LOAEC values, based on a lack of treatment-related effects, were 1000 and >1000 mg a.i./kg dw of soil, respectively. The active ingredient is considered to be non-toxic to earthworms up to a concentration of 1000 mg a.i./kg dw of soil.

Following 14 days of exposure, mortality was 2.5% in the nominal 31.3 mg a.i./kg dw soil treatment level and 0% in the control and 62.5-1000 mg a.i./kg dw soil treatment levels. By test termination, control worms had lost an average of 14.02% of their initial body weight, while worms in the nominal 31.3, 62.5, 125, 250, 500 and 1000 mg a.i./kg dw soil treatment levels lost an average of 12.08, 14.37, 15.74, 14.86, 19.13 and 17.01% of their initial body weight, respectively. No pathological abnormalities were observed.

This study is classified as **ACCEPTABLE** to the **U.S. EPA** and as **FULLY RELIABLE** to **PMRA and APVMA** as it is scientifically sound for an acute toxicity study with earthworms [850.6200].

Results Synopsis

Test Organism Size/Age(Mean Wt or Length): 405.2-497.6 mg (range of worms on Day 0)
Test Type (Flow-through, Static, Static Renewal): Not applicable

LC_{50}: >1000 mg a.i./kg dw soil	95% C.I.: N/A
EC_{50}: >1000 mg a.i./kg dw soil	95% C.I.: N/A
NOAEC: 1000 mg a.i./kg dw soil	
Probit Slope: N/A	95% C.I.: N/A
Endpoint(s) Affected: None	

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted following guidelines outlined in OECD Guideline for Testing of Chemicals, "Earthworm, Acute Toxicity Tests", No 207; and OECD Guideline for Testing of Chemicals, "Earthworm, Reproduction Test (*Eisenia fetida/Eisenia andrei*)", No 222. OPPTS guidance is only available for sub-chronic tests with earthworms. This study was conducted under 14-day acute conditions; therefore, the 850.6200 guidance is used for reference of environmental and culturing conditions. The following deviation from OPPTS 850.6200 was noted:

Earthworms were only acclimated to the artificial soil for 24 hours instead of the recommended 14 days.

This deviation does not impact the acceptability of the study.

COMPLIANCE: Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with the Good Laboratory Practice Standards as described in the U.S. EPA, Title 40 Code of Federal Regulations Part 160, Federal Register with the following exception: this study was been performed in accordance with the OECD Guidelines for Testing of Chemicals, Number 207, OECD: Paris, ISO 11268, part 1, 1993 and the Principles of Good Laboratory Practice Hungarian GLP Regulations: 9/2001 (III.30) EÜM-FVM joint decree of the Minister of Health and the Minister of Agriculture and Regional Development which corresponds to the OECD GLP, ENV/MC/CHEM (98) 17.

A. MATERIALS:

1. Test Material BAS 800 H (Saflufenacil)

Description: Not Reported

Lot No./Batch No. : COD-000515 (Batch Number)

Purity: 93.8%

Stability of compound under test conditions: Not Determined
(OECD recommends water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Storage conditions of test chemicals: Stored at room temperature.

Physicochemical properties of BAS 800 H.

Parameter	Values	Comments
Water solubility at 20 °C	2.1 g/L	pH 7
Vapor pressure	4.5×10^{-15} Pa	20°C
UV absorption	272 nm	pH1/pH7
pKa	Neutral	Ambient pH
Kow	Log P_{ow} 2.6	20°C

2. Test organism:

Species: *Eisenia fetida*
(EPA and OECD recommend Eisenia fetida andrei (Bouche). The earthworms should weigh 300-600 mg at the beginning of the test.)

Age at test initiation: Adult (at least two months old and had a clitellum)

Weight at study initiation: 405.2-497.6 mg (range of worms on Day 0)

Source: In-house cultures originally obtained from BASF AG (Limburgerhof, Germany)

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: The concentrations used in the definitive study were based on the results of a 14-day non-GLP range-finding study. Nominal concentrations in the range-finding study were 0 (negative and acetone solvent controls), 3.33, 10, 33.3, 100, 333 and 1000 mg a.i./kg dw soil. No mortalities occurred in the controls or treatment levels during the 14-day exposure period. Mean percent weight loss was 14.38 and 16.61% in the negative and solvent controls, respectively, and 19.13, 16.04, 11.76, 15.40, 17.49 and 21.54% in the nominal 3.33, 10, 33.3, 100, 333 and 1000 mg a.i./kg dw soil, respectively.

b. Definitive Study

1. Soil: Soil used in the definitive study was comprised of 5% sphagnum peat, 20% kaolin clay and 75% industrial sand. The pH was adjusted to 6.0±0.5 by addition of calcium-carbonate and the water content of the artificial soil before moistening was 0.99 g/100 g dry soil. The maximum water holding capacity was 45.35 g water/ 100 g soil dry weight.

Table 1: Physicochemical Properties of Natural Soil

Property	Value	Remarks

		Criteria
For natural soil: Texture: % sand % silt % clay Textural classification For artificial substrate (provide composition):	N/A 5% sphagnum peat 20% kaolin clay 75% industrial sand	The air-dry constituents were blended in the correct proportions and mixed thoroughly with deionised water. *Recommended testing medium is artificial soil consisting of a mixture of 68% of No. 70 mesh silica sand, 20% kaolin clay, 10 sphagnum peat moss, and 2% calcium carbonate, mixed and moistened to 35% by weight with deionized/distilled water.*
pH	Test initiation: 5.72-5.83 Test termination: 5.54-5.65	
Organic carbon (%)	N/A	The percentage of organic carbon was not reported; however the amount of peat was reduced from 10% to 5%, given the reported log Pow of BAS 800 H (2.6) and its potential influence on bioavailability.
Moisture (%)	Test initiation: 55.52% of the water holding capacity (WHC) Test termination: 56.16% of WHC	WHC of the test substrate = 45.38 g / 100 g soil dry weight

Table 2: Experimental Design

Parameter	**Detail**	**Remarks**
		Criteria
Acclimation: duration: conditions (state if same as the test conditions): health:	 24 Hours Same as test No disease or mortality reported	 *Earthworms should be acclimated at test temperature for 7 days.*

Parameter	Detail	Remarks *Criteria*
Soil [fresh or stored]	Prepared fresh	
Test Container material size amount of soil/substrate	 Glass 1 L 750 g wet weight	Test vessels were covered with transparent and punctured lids to allow gas exchange.
No. of replicates: per treatment group: per control:	 4 4	 *Recommended number of replicates include at least 3 and a control.*
No. of earthworms per treatment	40 worms per control and treatment level, equally divided among 4 replicates	*Recommended number of earthworms per treatment include a minimum of 30 plus a control; 10 per each of three replicates and a control.*
Solvents used or not (if yes report the name and concentration)	N/A; a solvent was not used	
Rates of application: nominal: measured:	 0 (control), 31.3, 62.5, 125, 250, 500 and 1000 mg a.i./kg dw soil Not Determined	*Earthworms should be exposed to at least five test concentrations, in geometric series, in which the ratio is between 1.5 and 2.0 mg of test chemical per kg (air-dry weight) of artificial soil.*
Reference chemical (if used) name: concentration:	 2-Chloroacetamide 2.5, 5, 10, 20, 40 and 80 mg a.i./kg dw soil	
Test conditions: Temperature Lighting conditions Moisture	 18.4-21.4°C Continuous illumination (~647 lux) 25.19-25.49% (average water content)	*Recommended temperature: 22 + 2°C* *Recommended lighting: Continuous illumination, with a light intensity of 400 lux* *Recommended relative humidity: above 85%*
Duration of the study	14 Days	

Parameter	Detail	Remarks *Criteria*
		Recommended duration of study is 28 days.

2. Observations:

Table 3: Observations

Parameters	Details	Remarks *Criteria*
Observation intervals	Days 7 and 14	*Recommended observation intervals are days 7, 14, 21, and 28.*
Parameters measured including the sublethal effects/toxicity symptoms	-Mortality -Percent Weight Change -Pathological Symptoms	*The test is usually not acceptable if more than 20% of control earthworms die or the total mean weight of control earthworms lose 20% or more of body weight.*
Were raw data included?	Yes	
Other observations, if any	None Reported	

II. RESULTS AND DISCUSSIONS

A. MORTALITY:

Following 14 days of exposure, mortality was 2.5% in the nominal 31.3 mg a.i./kg dw soil treatment level and 0% in the control and 62.5-1000 mg a.i./kg dw soil treatment levels. The resulting NOAEC and LC_{50} values were 1000 and >1000 mg a.i./kg dw soil, respectively.

In the 14-day toxic reference test with 2-chloroacetamide, mortality was 0% in the control and 2.5, 5 and 10 mg a.i./kg dw soil treatment levels and 10, 100 and 100% in the 20, 40 and 80 mg a.i./kg dw soil treatment levels, respectively. The study author reported NOAEC and LC_{50} (with 95% C.I.) values of 20 and 25.47 (23.20-28.43) mg a.i./kg dw soil, respectively.

Table 4: Effect of BAS 800 H on Mortality of *Eisenia fetida*

Nominal Treatment Levels (mg a.i./kg soil)	Observation Period			
	Day 7		Day 14	
	No Dead	% Mortality	No Dead	% Mortality
Negative Control	0	0	0	0
31.3	1	2.5	1	2.5
62.5	0	0	0	0
125	0	0	0	0
250	0	0	0	0
500	0	0	0	0
1000	0	0	0	0
NOAEC	1000 mg a.i./kg dw soil			
LOAEC	>1000 mg a.i./kg dw soil			
LC_{50}	>1000 mg a.i./kg dw soil			
Reference chemical % mortality: LC_{50}	2- Chloroacetamide- LC_{50}: 25.47 (23.20-28.43) mg a.i./kg dw soil			

B. SUB-LETHAL TOXICITY ENDPOINTS:

By test termination, control worms had lost an average of 14.02% of their initial body weight, while worms in the nominal 31.3, 62.5, 125, 250, 500 and 1000 mg a.i./kg dw soil treatment levels lost an average of 12.08, 14.37, 15.74, 14.86, 19.13 and 17.01% of their initial body weight, respectively. The study author reported no significant difference in biomass reduction as compared to the control in any treatment level with respective NOAEC and EC_{50} values of 1000 and >1000 mg a.i./kg dw soil.

No pathological abnormalities were observed.

In the 14-day reference test with 2-chloroacetamide, percent weight loss ranged from -20.54% in the 5 mg a.i./kg dw soil treatment level to -17.69% in the control in treatment levels containing surviving worms. Percent weight loss in the treatment levels did not differ significantly relative to the negative control.

Table 5: Sub-lethal Effect of BAS 800 H on *Eisenia fetida*. Values represent the treatment means

Treatment (mg a.i./kg soil) [indicate if measured or nominal conc. used]	Observation Period			
	Day 0		Day 14	
	Weight	% Loss	Weight	% Loss
Negative Control	462.9	N/A	397.8	14.02
31.3	456.2	N/A	401.0	12.08
62.5	440.9	N/A	377.9	14.37
125	442.9	N/A	373.3	15.74
250	470.0	N/A	400.1	14.86
500	460.3	N/A	372.4	19.13
1000	457.8	N/A	380.3	17.01
NOAEC	1000 mg a.i./kg dw soil			
LOAEC	>1000 mg a.i./kg dw soil			
EC_{50}	>1000 mg a.i./kg dw soil			
Reference chemical % mortality: LC_{50}	2-Chloroacetamide: % Weight Loss Range: -20.54% to -17.69%			

C. REPORTED STATISTICS:

For worm weight mean values and standard deviations were calculated for each treatment and each replicate at the start and at the end of the test. Statistical analysis on worm biomass was assessed by analysis of variance (ANOVA) Bonferroni t-Test and Dunnett's Test (α=0.05) by Toxstat software. Statistical analysis of worm mortality was assessed by analysis of variance (ANOVA) Fisher's Exact Test (α=0.05) by Toxstat software. The premises of ANOVA (at least homogeneity of variance) were tested by adequate statistics.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): Toxicity values for mortality were visually determined because mortality occurred in one treatment level only and was only 2.5%. Prior to determining the toxicity values for percent weight loss, the reviewer tested the replicate data for normality using the Chi-square and Shapiro-Wilks tests and for homogeneity of variance using the Hartley and Bartlett's tests via Toxstat statistical software. The data met these assumptions of ANOVA; therefore, the NOAEC value was determined using the parametric Dunnett's and Williams' tests. All analyses were conducted using the nominal concentrations.

LC_{50}: >1000 mg a.i./kg dw soil 95% C.I.: N/A
EC_{50}: >1000 mg a.i./kg dw soil 95% C.I.: N/A
NOAEC: 1000 mg a.i./kg dw soil

Probit Slope: N/A 95% C.I.: N/A
Endpoint(s) Affected: None

E. STUDY DEFICIENCIES:

There were no study deficiencies.

F. REVIEWER'S COMMENTS:

The reviewer's results were identical to those of the study author.

In order to prepare the treatment levels, ventilated and moistened mixtures of saflufenacil and fine quartz sand were added to artificial soil and homogenized using a laboratory mixer. The control was treated with the same amount of sand as the test item groups. For two replicates of each test concentration, a mixture of 22g fine quartz sand and quantity of test item corresponding to 1650 g wet weight of artificial soil were mixed with 1287.9 g wet artificial soil.

The in-life portion of the definitive toxicity test was conducted from November 9 to November 23, 2006.

G. CONCLUSIONS:

The study is classified as ACCEPTABLE to U.S. EPA and as FULLY RELIABLE to PMRA and APVMA The NOAEC, EC_{50} and LC_{50} values, based on a lack of treatment-related effects, were 1000, >1000 and >1000 mg a.i./kg dw soil, respectively.

LC_{50}: >1000 mg a.i./kg dw soil 95% C.I.: N/A
EC_{50}: >1000 mg a.i./kg dw soil 95% C.I.: N/A
NOAEC: 1000 mg a.i./kg dw soil
Probit Slope: N/A 95% C.I.: N/A
Endpoint(s) Affected: None

III. REFERENCES:

OECD Guideline for Testing of Chemicals, Section 2., "Earthworm, Acute Toxicity Tests", No.: 207, adopted 4 April 1984.

ISO International Standard, Soil quality- Effects of pollutants on earthworms (*Eisenia fetida*), No.: 11268-1, Part 1., adopted 1993.

ISO International Standard, Soil quality- Determination of the water retention characteristic- Laboratory methods, No.: 11274, adopted 1998.

ISO International Standard, Soil quality- Determination of pH, No.: 10390, adopted 2005.

OECD Guideline for Testing of Chemicals, "Earthworm Reproduction Test (*Eisenia fetida/ Eisenia andrei*), No.: 222, adopted 13 April 2004.

Hungarian Good Laboratory Practice Regulations: 9/2001 (III. 30) EÜM-FVM joint decree of the Minister of Health and

the Minister of Agriculture and Regional Development which corresponds to the OECD GLP ENV/MC/CHEM (98) 17.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Mean % weight loss (Days 0-14); mg a.i./kg dw soil
File: 7927wl Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.876	6.776	10.696	6.776	1.876
OBSERVED	0	8	9	11	0

Calculated Chi-Square goodness of fit test statistic = 6.8752
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Mean % weight loss (Days 0-14); mg a.i./kg dw soil
File: 7927wl Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 209.247

W = 0.961

Critical W (P = 0.05) (n = 28) = 0.924
Critical W (P = 0.01) (n = 28) = 0.896

Data PASS normality test at P=0.01 level. Continue analysis.

Mean % weight loss (Days 0-14); mg a.i./kg dw soil
File: 7927wl Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 18.64
Closest, conservative, Table H statistic = 216.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 7, df (# reps-1) = 3
Actual values ==> R (# groups) = 7, df (# avg reps-1) = 3.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

```
Mean % weight loss (Days 0-14); mg a.i./kg dw soil
File: 7927wl        Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance
-------------------------------------------------------------------------------

Calculated B statistic =    6.73
Table Chi-square value =   16.81  (alpha = 0.01)
Table Chi-square value =   12.59  (alpha = 0.05)

Average df used in calculation  ==>   df (avg n - 1) =   3.00
Used for Chi-square table value ==>   df (#groups-1) =   6
-------------------------------------------------------------------------------

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
      used to calculate the B statistic (see above).

Mean % weight loss (Days 0-14); mg a.i./kg dw soil
File: 7927wl         Transform: NO TRANSFORMATION

                                ANOVA TABLE
-------------------------------------------------------------------------------

SOURCE             DF              SS                MS              F
-------------------------------------------------------------------------------
Between             6             123.379          20.563          2.064

Within (Error)     21             209.247           9.964
-------------------------------------------------------------------------------
Total              27             332.626
-------------------------------------------------------------------------------

  Critical F value =   2.57  (0.05,6,21)
  Since  F < Critical F  FAIL TO REJECT  Ho:All groups equal


Mean % weight loss (Days 0-14); mg a.i./kg dw soil
File: 7927wl         Transform: NO TRANSFORMATION

      DUNNETTS TEST   -   TABLE 1 OF 2            Ho:Control<Treatment
-------------------------------------------------------------------------------
                              TRANSFORMED    MEAN CALCULATED IN
GROUP    IDENTIFICATION          MEAN          ORIGINAL UNITS    T STAT  SIG
----- --------------------    ----------    ------------------  ------  ---
  1            neg control      14.023            14.023
  2                   31.3      12.080            12.080          0.870
  3                   62.5      14.365            14.365         -0.153
  4                    125      15.738            15.738         -0.768
  5                    250      14.857            14.857         -0.374
  6                    500      19.130            19.130         -2.288
  7                   1000      17.008            17.008         -1.337
-------------------------------------------------------------------------------
Dunnett table value =  2.46     (1 Tailed Value, P=0.05,  df=20,6)
```

Mean % weight loss (Days 0-14); mg a.i./kg dw soil
File: 7927wl Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			
2	31.3	4	5.491	39.2	1.943
3	62.5	4	5.491	39.2	-0.342
4	125	4	5.491	39.2	-1.715
5	250	4	5.491	39.2	-0.835
6	500	4	5.491	39.2	-5.107
7	1000	4	5.491	39.2	-2.985

Mean % weight loss (Days 0-14); mg a.i./kg dw soil
File: 7927wl Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	4	14.023	14.023	13.051
2	31.3	4	12.080	12.080	13.051
3	62.5	4	14.365	14.365	14.365
4	125	4	15.738	15.738	15.298
5	250	4	14.857	14.857	15.298
6	500	4	19.130	19.130	18.069
7	1000	4	17.008	17.008	18.069

Mean % weight loss (Days 0-14); mg a.i./kg dw soil
File: 7927wl Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	13.051				
31.3	13.051	0.435		1.72	k= 1, v=21
62.5	14.365	0.153		1.80	k= 2, v=21
125	15.298	0.571		1.83	k= 3, v=21
250	15.298	0.571		1.84	k= 4, v=21
500	18.069	1.813		1.85	k= 5, v=21
1000	18.069	1.813		1.85	k= 6, v=21

s = 3.157
Note: df used for table values are approximate when v > 20.